D. C., H. L. Sanford, August 23, 1919; *E. repens* L., Mattituck, N. Y., Roy Latham, July 18, 1920; wintergreen, Albany, N. Y., E. P. Felt, May 16, 1922; laurel, Washington, D. C., W. B. Wood, July 26, 1927, and R. G. Cogswell, May 24, 1928; blueberry (in greenhouse), Washington, D. C., September 2, 1932; *E. repens* L., Prince Edward Island, New Brunswick, and Nova Scotia, Canada, intercepted at Boston, Mass., by J. T. Beauchamp, W. J. Ehinger, and E. Hodson, May 23, 1939, to May 4, 1943; *E. repens* L., Sligo Park, Silver Spring, Md., Louise M. Russell, November 6, 1943 (including holotype).

In the lot containing the holotype, 2 third-stage specimens lack submedian mesothoracic and metathoracic setae; 2 apparently second-stage insects lack mesothoracic setae, but a metathoracic one is suggested on one half of one specimen and on each half of the other; in 10 first-stage insects, mesothoracic and metathoracic setae are present but are much smaller than the cephalic ones. The vasiform orifice of the third-stage specimens is similar to that of the pupae.

This species was abundant on some samples of *Epigaea* examined by the writer. It is rather similar to *A. myricae*.

Aleuroplatus bignoniae, n. sp.

Differing from *A. plumosus* as follows: Submedian mesothoracic and metathoracic setae absent; caudal setae slightly farther apart than eighth abdominal ones and nearer to submarginal teeth than to orifice. Two pairs of central subdorsal minute setal bases on prothorax, abdominal ones usually nearer to inner than to central subdorsal disk pores except on segment 3. One distinctly submedian pair of dorsal disk pores on each of abdominal segments 1–7; subdorsal abdominal ones unusually numerous, more or less grouped in inner, central, and outer subdorsum, the outer ones sometimes nearly indistinguishable from submarginal ones; abdominal segment 3 with 8–13 pairs, segment 4 with 5–9 pairs, segment 5 with 4 or 5 pairs, segment 6 with 2–4 pairs, segment 7 with 1 pair. Vasiform orifice around 56μ long and 48 wide; tongue located at end of orifice.

Type.—U.S.N.M. No. 56957. Brooksville, Fla., from *Bignonia*.

Described from several unmounted specimens and six mounted ones from *Bignonia* sp., Brooksville, Fla., H. L. Sanford, Feb. 11, 1922.

The one available third-stage specimen lacks meso- and metathoracic setae, and has the vasiform orifice similar to that of the pupae.

ZOOLOGY.—*Tests indicating absence of progesterone in certain avian ovaries.*[1]

Oscar Riddle and James Plummer Schooley,[2] Carnegie Institution of Washington, Cold Spring Harbor, N. Y.

Histological evidence of the presence of luteal tissue in the ovary of fowl, and of some other birds, has been frequently asserted and perhaps still more frequently denied. This subject was treated extensively by Fell (1925). In reptiles, however, histological studies seem to have demonstrated the presence of a typical corpus luteum in some species and its absence in others. In certain viviparous lizards both macroscopic and microscopic evidence of corpora lutea was noted by Hett (1924), Weekes (1934), and Cunningham and Smart (1934). The last-named authors also noted the absence of corpora in oviparous lizards. Clausen (1935) briefly reported important observations on the presence, and on effects of removal (total ovariectomy), of "luteal" bodies in viviparous snakes. Fraenkel and Martins (1938) noted the presence, in pregnant viviparous snakes, of bodies indistinguishable from the corpora lutea of mammals and further showed that these corresponded in number to the ova or embryos present in the oviducts. At this stage in the development of the subject a short abstract of results of the present study was published. (Riddle and Schooley, 1938a).

Further morphological studies on the corpora of viviparous snakes have been made by Rahn (1938; 1939) and Fraenkel and Martins (1939; 1940). Porto (1941) made crude ethanol extracts of such corpora

[1] Received June 24, 1944.

[2] Now director of Endocrine Laboratories, Difco Laboratories, Inc., Detroit, Mich.

and showed that they contained progesterone. It should be noted that Porto's tests were made by *subcutaneous* injection into immature rabbits to which 10 I.U. of estradiol benzoate had been administered daily for eight days. Though relatively large amounts of progesterone are required for detection following subcutaneous injection, an extract from only 2.9 gm of corpora from pregnant snakes was shown to contain progesterone. Slightly preceding this group of studies, McGinty, Anderson, and McCullogh (1938; 1939) developed a highly sensitive method by which as little as 0.25 to 1 μg of crystalline progesterone may be detected. That method made it practicable to carry out the present study, since fowl and pigeon ovaries could be expected to contain only minute amounts of progesterone. In Corner's laboratory Haskins (1939) observed that as little as 0.25 gamma of progesterone, also the amount present in 0.2 cc serum from a pregnant guinea-pig, may be detected by the McGinty test. Later, Haskins (1940) reported important studies in which mitotic counts in the uterine epithelium were utilized to increase the sensitivity of the test, and also to make it usable for the quantitative assay of progesterone.

Besides contributing to our growing knowledge of progesterone production in oviparous and viviparous vertebrates, it was hoped that the results of the present study might incidentally provide information bearing on the role of progesterone in the induction of broodiness in lower vertebrates. Noble, Kumpf, and Billings (1938) noted that progesterone, like prolactin, has the ability to induce broody behavior in normal and castrate jewelfish; and Riddle and Schooley (1938b) observed that some male and female ring doves could be made completely broody within 2 to 5 days by temporary implantation of pellets of progesterone. If bird ovaries were found to contain much progesterone this would provide at least a possibility that this hormone may participate in the onset or regulation of broodiness; if bird ovaries were shown to contain no progesterone any physiologically significant role of this hormone in broodiness would be rendered doubtful or excluded. This question has not been definitely answered, however, by the present study. It has been shown that properly conducted tests on three samples of bird ovaries, all of which were suitable for test (since they contained ovulated follicles in various early stages of regression and a few growing follicles), failed to show the minute amount of progesterone that is detectable by the McGinty test.

MATERIAL

Tests were made on a sample of rat ovaries, a sample of pigeon ovaries, and on two samples of fowl ovaries. Each of the two samples of fowl ovaries, prepared for study of their progesterone content, was derived from three hens belonging to three different breeds (Rhode Island Red, Plymouth Rock, White Rock); in all these breeds the hens are usually capable of becoming broody. These six laying hens, whose egg-laying records were accurately known for the previous five days, were removed from fenced-in "runs" on an adjacent farm to similar "runs" at the laboratory three days before their ovaries were removed; trap-nest records were continued to the time of killing for samples. During this 3-day interval one group of three hens received no treatment except such as was incidentally connected with their change of habitat and food; though these changes usually diminish egg production, laying was not immediately suppressed in either of these three birds and both unovulated follicles and ovulated follicles in various stages of regression were found in each of the three ovaries. Extract *A* was prepared from the ovaries of this group. Two of the three hens of the other group laid irregularly during the total of eight days for which trap-nest records were kept; each of these hens received 1,000 units (3.33 mg) of luteinizing hormone from pregnancy urine daily during the last three days, and although only one of them was definitely known to lay during this period (two eggs were laid outside the trap-nest) rather recently emptied follicles were found at autopsy in all cases. Extract *B* was prepared from the ovaries of this

group. In all cases the walls of all ova (follicles) more than 2 mm diameter were slit open and the yolk contents eliminated from the sample. Thus 16.8 gm of ovarian tissue, including the thick walls of unovulated and of recently ovulated follicles, was the starting point for extract *A*, and 12.1 gm the source of extract *B*. These two samples of ovarian tissue were then subjected to Allen's (1932) method for the preparation and purification of progesterone. Each extract was injected into two rabbits, one of which (lower ones on table 1), however, received more than two-thirds of the preparation. The McGinty method was followed closely with the exception that any progesterone contained in our extracts was not in solution in peanut oil but in unseparated fatty material (soluble in methyl alcohol) from the bird's own ovary. These tests were made during May-June.

The pigeon ovaries selected for extraction and study were obtained within 20 hours after the ovulation of the second ovum of the clutch. This period is practically coincident with the onset of broodiness in pigeons, and the two ovulated follicles had, respectively, undergone 64 and 20 hours of regression.

Most of the rabbits used were of a large breed, New Zealand White. All were less than 2.0 months old when priming with estrone was started.

RESULTS

Table 1 gives details of the dosages used and the results of treatments. The data indicate that the simultaneous presence of some lipoid material other than progesterone—such as is contained in a crude methyl alcohol extract of brain tissue—did not measurably affect the sensitivity of the McGinty test for progesterone. Though the rabbits used gave positive tests with 1 μg progesterone, with or without admixture of the brain extract, two rabbits were wholly negative to extracts (*A* and *B*) from approximately 10 gm of prepared fowl ovary; two other rabbits were wholly negative to slightly less than one-half that quantity of the extracts. A crude extract from only 120 mg of luteinized rat ovary gave a reaction equal to that obtained with 1 or 2 μg of progesterone. The "plus" signs (+ to +++) of the table are not necessarily the same as those of previous workers; they represent degrees of stimulation observed in our tests.

Table 1.—Data Relating to Tests for Progesterone in Extracts of Ovaries of Fowl, Pigeons, and Rats by Injection into Uteri of Immature Rabbits

Rabbit weight	Material and quantity in cc injected into—				Progestational proliferation	
	Right horn		Left horn		Right	Left
		cc		cc		
963	4 μg progesterone	0.2	Sesame oil	0.2	+++	−
900	Extr. pigeon ovary[1]	0.2	Crude extr. brain[2]	0.2	−	−
1775	Part extract *A*[3]	0.25	2 μg progesterone	0.1	−	++
1695	Part extract *B*[4]	0.2	1 μg progesterone	0.05	−	+
1075	Remainder extract *A*[3]	0.6	None		−[5]	−
1090	Remainder extract *B*[4]	0.5	None		−[5]	−
1185	1 μg prog. + brain extr.	0.2	1 μg progesterone	0.1	++	+
1760	4 μg. prog.[6] + brain extr.	0.2	4 μg progesterone	0.2	+	+++
1250	1 μg progesterone	0.1	Extr. rat ovary[7]	0.2	++	++

[1] Crude methyl alcohol extract (73 mg) of two pigeon ovaries (1.0 gm).

[2] Similar crude methyl alcohol extract (50 mg) of brain of pigeons.

[3] The smaller dose (0.25 cc) represented 30 percent, and the larger dose (0.6 cc) 70 percent, of the total "progesterone fraction" obtained from 16.8 gm of fowl ovaries.

[4] The smaller dose (0.2 cc) represented 29 percent, and the larger dose (0.5 cc) 71 percent, of the total "progesterone fraction" obtained from 12.1 gm of ovaries from treated fowl.

[5] The isolated loop of the uterus was distended with purulent fluid.

[6] A considerable fraction of this material was lost at injection.

[7] The injected material (not weighed) was a crude, methyl alcohol extract of 120 mg of rat ovary heavily luteinized with a preparation from pregnancy urine.

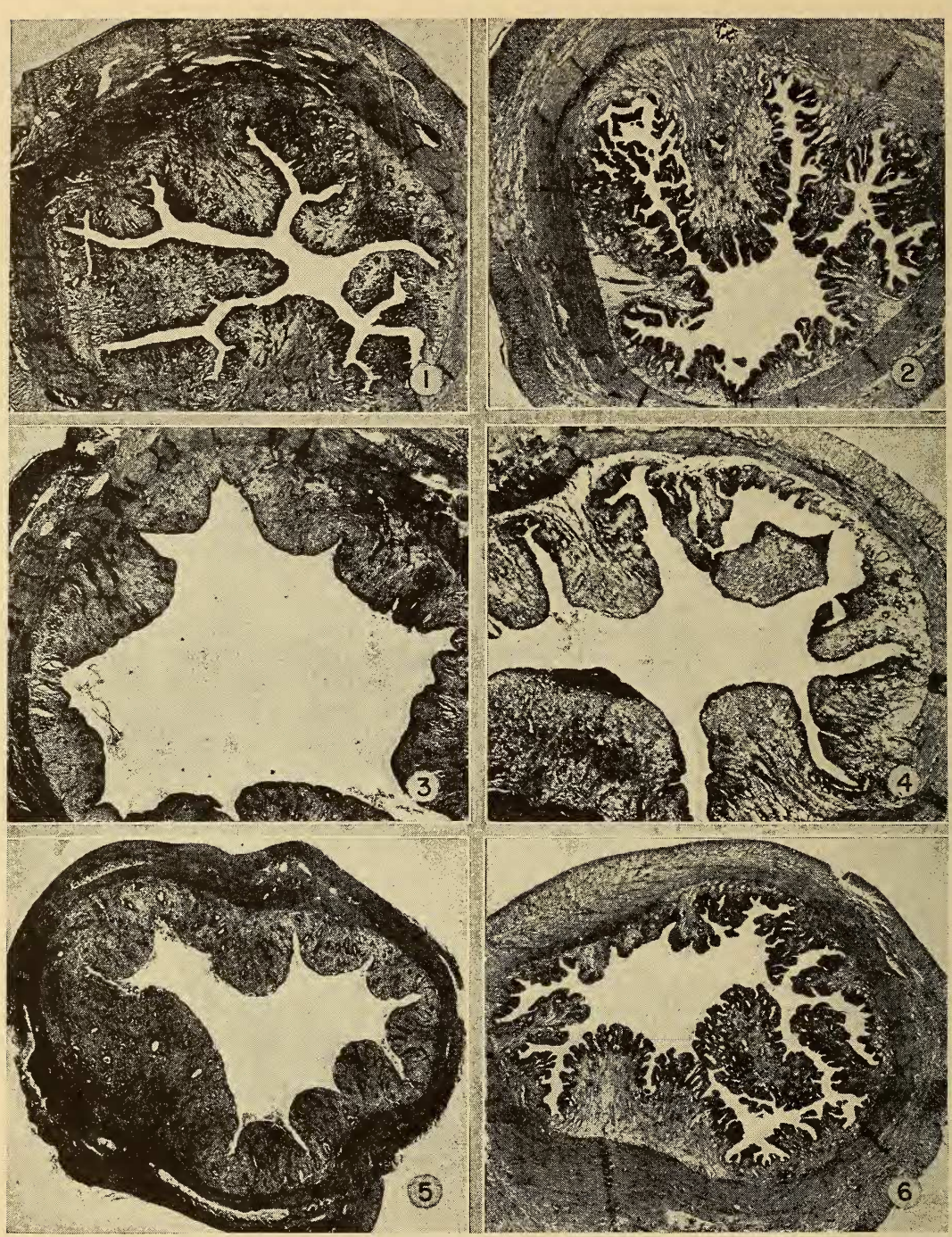

Fig. 1.—Left horn of rabbit uterus (control) injected with 0.2 cc sesame oil. No progestational proliferation. Fig. 2.—Right horn of rabbit uterus injected with 1μg progesterone+brain extract (0.2 cc). Well-marked progestational proliferation. Fig. 3.—Right horn of rabbit uterus injected with extract *A* (0.6 cc) from 11.7 gm fowl ovaries. Distention but no progestational proliferation. Fig. 4.—Right horn of rabbit uterus injected with extract *B* (0.5 cc) from 8.6 gm of ovaries from fowl treated for three days with human pregnancy urine. Distention but no progestational proliferation. Fig. 5.—Right horn of rabbit uterus injected with extract *B* (0.2 cc) from 3.5 gm of ovaries from fowl treated for three days with human pregnancy urine. No progestational proliferation. Fig. 6.—Left horn of rabbit uterus injected with crude extract of 120 mg rat ovary luteinized with human pregnancy urine. Well-marked progestational proliferation.

The condition of several uteri following treatment with extracts of various types of ovaries is shown in Figs. 2–6. A control uterus, injected with sesame oil only (Fig. 1) and three of the uteri treated with extracts *A* and *B* (Figs. 3–5) show no progestational proliferation. A uterus treated with 1 μg progesterone+brain extract (Fig. 2), and another treated with extract of rat ovary (Fig. 6), show such proliferation clearly.

DISCUSSION

The studies of Fraenkel and Martins, and of Porto, seem to have demonstrated that corpora lutea and progesterone (i.e., a substance able to induce progestational proliferation) are produced in some viviparous reptiles. It was thus made evident that vertebrates both lower and higher than birds are capable of producing luteal cells and progesterone. The fact that some *families* of lizards and snakes contain both oviparous and viviparous species—and that the viviparous forms are presumably derived from oviparous ancestors—suggests that a *latent* capacity to produce this hormone may be widespread among exclusively oviparous forms, such as birds. Indeed, it may now be regarded as probable that a wide distribution of that latent capacity was a prerequisite for the origin and success of intrauterine embryonic development in several unrelated genera of reptiles and in (early) mammals. Perhaps only special endocrine and oviducal states or conditions can convert this latent capacity into the actual formation of luteal cells (and of progesterone production in detectable amounts) in oviparous birds, and certainly not all these states or conditions were subjected to test in this investigation. The present study is a contribution to this problem since, for the first time, the ovaries of two oviparous species have been tested for the presence or absence of progesterone. Tests on other species and reproductive states will require several additional investigations. All that is claimed for the present study is that the ovaries utilized by us were shown to contain either no progesterone or an amount which is relatively insignificant in comparison with that found in ovaries of mammals.

The apparent ability of progesterone to induce broodiness in some fishes (Noble, Kumpf, and Billings, 1938) and birds (Riddle and Schooley, 1938b; Riddle and Lahr, 1944), and its similar ability to initiate maternal behavior in rats (Riddle, Lahr, and Bates, 1942), provided a special reason for interest in the outcome of our search for progesterone in the ovaries of fowl and pigeons. If progesterone is a substance directly concerned in the release of broodiness, and not merely one of a variety of substances having ability to cause the pituitary to release the directly effective hormone (Riddle, Lahr, and Bates, 1942), it should be possible to obtain detectable amounts of progesterone from the bird ovary. The absence of such amounts of that hormone in the present tests provides an item of evidence, inconclusive though it is, that progesterone is not directly involved in the initiation of broody behavior.

SUMMARY

The sensitive McGinty test showed that a crude extract of 120 mg of luteinized rat ovary contained more than sufficient progesterone to produce definite progestational proliferation in the uterus of an immature rabbit.

Partly purified extracts from 11.7 gm (also from 5.1 gm) of ovarian tissue from three laying hens, and similar extracts from 8.6 gm (also from 3.5 gm) of ovaries of three such hens treated over three days with 3,000 units of luteinizing hormone from pregnancy urine, did not contain an amount of progesterone detectable by the McGinty test. A test made with a crude extract from two pigeon ovaries (1.0 gm) taken at 20 hours after ovulation of the second egg of the clutch was likewise negative.

Since 0.25 to 1.0 μg of crystalline progesterone, and also the amount of progesterone present in 0.2 cc of serum from a pregnant guinea-pig, are detectable by this method, it is concluded that fowl and pigeon ovaries in the reproductive phases tested by us contain either no progesterone or a relatively insignificant amount in comparison with that found in ovaries of mammals and some viviparous snakes.

It is noted that these results have bearing upon questions involved in the numerous independent origins of viviparity in families of higher vertebrates, and also upon the possibility that progesterone participates in the normal induction of broodiness in birds.

LITERATURE CITED

ALLEN, W. M. *The preparation of progestin.* Journ. Biol. Chem. **98**: 591. 1932.

CLAUSEN, H. J. *The effects of ovariotomy and hypophysectomy on parturition in snakes.* Anat. Rec. (Suppl.) **64**: 88. 1935.

CUNNINGHAM, J. T., and SMART, W. A. M. *The structure and origin of corpora lutea in some of the lower vertebrates.* Proc. Roy. Soc. London **116B** (798): 258. 1934.

FELL, H. B. *The relationship of the "luteal" cells of the ovary of the fowl to the tissue occupying the atretic and discharged follicles, and the question of the homology of the latter tissue and the mammalian corpus luteum.* Quart. Journ. Micr. Sci. **69**: 591. 1925.

FRAENKEL, L., and MARTINS, T. *Sur le corp jaune des serpents vivipares.* Compt. Rend. Soc. Biol. **127**: 466. 1938.

———. *Estudos sobre a fisiologia sexual das serpents.* Mem. Inst. Butantan **13**: 393. 1939.

——— and MELLO, R. F. *Studies on the pregnancy of viviparous snakes.* Endocrinology **27**: 836. 1940.

HETT, J. *Das corpus luteum der Zauneidechse* (Lacerta agilis). Zeit. mikr. Anat. Forsch. **1**: 41. 1924.

HASKINS, A. L. JR. *Assay of progesterone by intra-uterine application in the rabbit.* Proc. Soc. Exp. Biol. and Med. **42**: 624. 1939.

———. *Modification of the intrauterine assay method for progesterone.* Endocrinology **27**: 983. 1940.

MCGINTY, D. A., ANDERSON, L. P., and MCCULLOGH, N. B. *Effect of local application of progesterone on the rabbit uterus.* Amer. Journ. Physiol. (Proc.) **123**: 133. 1938.

———. *Effect of local application of progesterone on the rabbit uterus.* Endocrinology **24**: 829. 1939.

NOBLE, G. K., KUMPF, K. F., and BILLINGS, V. N. *The induction of brooding behavior in the jewel fish.* Endocrinology **23**: 353. 1938.

PORTO, A. *Sobre a presenca de progesterona no corpo amarelo de serpentes ovovivipares.* Mem. Inst. Butantan **15**: 27. 1941.

RAHN, H. *The corpus luteum of reptiles.* Anat. Rec. (Suppl.) **72**: 55. 1938.

———. *Structure and function of placenta and corpus luteum in viviparous snakes.* Proc. Soc. Exp. Biol. and Med. **40**: 381. 1939.

RIDDLE, O., and LAHR, E. L. *On broodiness of ring doves following implants of certain steroid hormones.* Endocrinology **35**. 1944.

——— and BATES, R. W. *The role of hormones in the initiation of maternal behavior in rats.* Amer. Journ. Physiol. **137**: 299. 1942.

RIDDLE, O., and SCHOOLEY, J. P. *Tests indicating absence of progesterone in fowl ovary.* Anat. Rec. (Suppl.) **62**: 59. 1938a.

———. *Hormonal basis of maternal behavior and broodiness.* Carnegie Inst. Washington Year Book **37**: 56. 1938b.

WEEKES, H. C. *On the distribution, habitat and reproductive habits of certain European and Australian snakes and lizards with particular regard to their adoption and adaptivity.* Proc. Linn. Soc. New South Wales **59**: 380. 1934.

PROCEEDINGS OF THE ACADEMY AND AFFILIATED SOCIETIES

CHEMICAL SOCIETY

559TH MEETING

The 559th meeting was held in the Auditorium of the Cosmos Club at 8:15 P.M. on Thursday, January 13, 1944. The retiring President, STERLING B. HENDRICKS, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, spoke on *Polymer chemistry of silicates, borates, and phosphates.* This address was published in this JOURNAL **34**(8): 241-251. 1944.

560TH MEETING

The 560th meeting was held in the Auditorium of the Cosmos Club at 8:15 P.M. on Thursday, February 10, 1944. Donald B. Keyes, professor of chemical engineering, University of Illinois, and chief of the Chemical Industries Branch of the Office of Production Research and Development, War Production Board, spoke on *The chemical side of the Davis-Keyes mission to London.*

561ST MEETING

The 561st meeting was held in the Statler Hotel on Thursday, March 9, 1944, at 7:30 P.M. This was the annual dinner meeting of the Society. The Hillebrand Prize for 1943 was awarded to B. H. NICOLET, Bureau of Dairy